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FINAL

No.2

REPORT/ON

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CONTRACT NO DA-92-557-FEC-34462

INCLUSIVE DATES 15 December, 1961 TO 14 December, 1962

SUBJECT OF INVESTIGATION

EFFECTS OF RADIATIONS ON CELL DIVISION

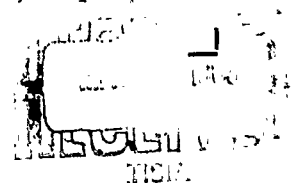
AND

CHROMOSOMES IN ANIMALS

RESPONSIBLE INVESTIGATOR

Dr. Sajiro Makino

Professor of Zoology, Makino Laboratory
Zoological Institute, Faculty of Science
Hokkaido University, Sapporo, Japan.



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Hokkaido University (Japan)
EFFECTS OF RADIATIONS ON CELL DIVISION AND
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Final report No. 2, 15 Dec 61 - 14 Dec 62. 9 p.
incl. tables, 6 refs.
(Contract DA-92-557-FEC-34462)Unclassified report.

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Pupae were irradiated at various stages and

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Spermatogenesis-
Drosophila
4. Gamma-Rays
Paramesium

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ABSTRACT OF THE FINAL REPORT NO. 2.

Effects of radiations on cell division and chromosomes in animals were studied from various stand points of view. Firstly, effects of X- and beta- irradiations on grasshopper spermatocytes were investigated using various chemicals with special regards to the radioprotection, but the result is not remarkable at present. Secondly, effects of single or repeated X-irradiation on chromosomes of human *in vitro* cell line were studied. Results were as follows: a) after a single dose irradiation, normal diploidy was retained for as long as 12 passages over 100 days. Variation of chromosome number occurred in only earlier passage and b) six sublines were produced by the repetition of X-irradiation. About fifty percent of cells in each sublines showed chromosome aberrations. Thirdly, genetic studies of anomalies in the mouse populations whose ancestors have received X-irradiation were investigated: pregnancy rate, mortality of foetuses, and litter size in females which were mated with irradiated males were observed. Results showed that a) pregnancy rate was same as control, b) number of moles increased remarkably, and c) litter size decreased slightly. Fourthly, effects of X-rays on the spermatogenesis of *Drosophila* were studied. Pupae were irradiated at various stages and emergence rate, hatchability of eggs, and spermatogenic chromosomes were observed. Results were as follows: a) the rate of emergence was almost same as control, b) hatchability of eggs showed extremely low percentage, and c) more than fifty percent of cells showed chromosome aberrations. Fifthly, effects of gamma-irradiation on mating type of *Paramecium* were studied using *P. polycarum* which has the binary mating type. Many new clones were obtained by the application of gamma-irradiation. These clones are very useful to analyse the genetic behaviour of *Paramecia* and further studies are in progress. Sixthly, immunological response of lethally irradiated mice which receive injection of heterologous spleen cells was studied. Histological observations on spleens were carried out as a supplemental experiment. Seventhly, effects of X-irradiation on the pregnancy rate in mice received polyovulation treatment were investigated. It was concluded that in order to facilitate the implantation of hormone-induced polyovulated eggs, 25 X-irradiation is effective.

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Professor of Zoology
Makino Laboratory
Zoological Institute
Faculty of Science
Hokkaido University
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<u>CONTENT</u>	<u>PAGE</u>
I. Results obtained to date	1
1. Effects of X-and beta-irradiations on germ-cells of grasshopper, with special regards to the behavior of the chromosomes	1
2. Effects of repeated X-irradiation on chromosome morphology of mammalian cells <u>in vitro</u>	1
a. A chromosome study in a non-treated and an irradiated human <u>in vitro</u> cell line	
b. A chromosomal survey on the repeatedly irradiated human cell lines <u>in vitro</u>	
3. Genetic studies of anomalies in the mouse population whose ancestors have received X-irradiations	2
a. Malformation in mouse embryos derived from normal females mated with X-irradiated males	
b. Malformation in mouse embryos derived from irradiated pregnant females	
c. Sensitivity of immature mouse germ cells to X-rays administered at various developmental stages	
d. Pregnancy and litter size of females mated with irradiated adult mice at various time in relation to the post-radiation effects	
4. Effects of X-rays on the spermatogenesis of <u>Drosophila virilis</u>	4

a. Emergence rate of pupae irradiated at various stages after pupation	
b. Cytological studies on the effects of X-irradiation on the germ cells of pupae in <u>Drosophila virilis</u>	
5. Effects of ^{GAMMA} X-irradiations on mating type of <u>Paramecium</u>	5
6. Immunological response of lethally irradiated mice which received injection of heterologous spleen cells	6
7. Effects of X-rays on the pregnancy rate in mice received polyovulation treatment	7
a. Induced polyovulation and super pregnancy in mature mice	
b. Induced polyovulation in mature golden hamster	
c. Effects of X-irradiation upon developing polyovulated mouse eggs	
II. List of papers published and in press	9

I. RESULTS OBTAINED TO DATE

1. EFFECTS OF X-AND BETA-IRRADIATIONS ON GERM-CELLS OF GRASSHOPPERS, WITH SPECIAL REGARDS TO THE BEHAVIOR OF THE CHROMOSOMES

Studies of this project have been performed using X- and beta- irradiation in combination with various compounds, radioprotection and radiomimetic agents, in order to assess the findings so far obtained in former year and to get any new findings. It had been tried to use dinitrophenol, RNA, and 5-bromouracil, as the treating agent, however, results were not remarkable by means of the phase cinematography. Accordingly, the effects of 5-bromouracil on the chromosomes of grasshopper-spermatocytes were observed by fixed and stained preparations and further investigations of this project are continued using the tissue culture materials. Results of a preliminary experiment of 5-bromouracil were as follows: 1) chromosomes of first and second spermatocytes were observed at 20, 60, minutes, 2, 4, 6, 12, 24, 36, and 48 hours after treatment, 2) most remarkable aberration was the chromosome deletion. 3) these deletions were observed only in the chromosomes of the second spermatocytes, mainly during the period of 20 and 60 minutes after treatment.

2. EFFECTS OF REPEATED X-IRRADIATION ON CHROMOSOME MORPHOLOGY OF MAMMALIAN CELLS IN VITRO

a. A chromosome study in a non-treated and an irradiated human in vitro cell line: In a non-treated and an irradiated human fibroblast cell lines of foetal lung origin, chromosome conditions were studied in the course of serial passages. The primary culture was started from lung cells derived from a four-month-old female foetus. Subculture was made from 5- to 10-day-old culture. The culture was separated into two groups at the third passage. Irradiation experiments were undertaken with one of the two groups of chemicals at the third passage. The culture was exposed to an X-irradiation (200r) and these irradiated cells were subcultured successively through the routine procedures for 12 passages. The other culture groups remained

non-irradiated and had been maintained for 47 passages. Chromosome analyses have been done through serial passages as shown in Tables 1 and 2. Normal diploidy and normal karyotype were retained in cells from the non-treated line until the 47th passage for 340 days (Table 1). The frequency of tetraploid and other aberrant cells suddenly increased just prior to the beginning of cell degeneration in later passages. In the irradiated cell line normal diploidy was retained for as long as 12 passages over 100 days. Polyploidy and variations of the chromosome number occurred in cell populations of earlier passages. (Table 2).

b. A chromosomal survey on the repeatedly irradiated human cell lines in vitro: Six sublines were produced by the repetition of X-irradiation of 200r serially at about one month interval. Chromosome analysis of these sublines has been carried out with special regards to the chromosome aberrations in relation to the radiosensitivity and resistancy of cells. Stained preparations were made from materials which were fixed 3, 5, 7 and 10 days after irradiation. As the chromosome counts are summarized in Table 3, about fifty percent of metaphase plates showed at least one chromosome aberrations; fragment, breakage, translocation, dicentrics, and endoreduplication etc. A tendency of decreasing the incidence of cell injuries according to the addition of culture passages, might suggest the radioresistancy of the irradiated strain.

3. GENETIC STUDIES OF ANOMALIES IN THE MOUSE POPULATION WHOSE ANCESTORS HAVE RECEIVED X-IRRADIATION

a. Malformation in mouse embryos derived from normal females mated with X-irradiated males: dd x C3A mice were used for this study. Male mice of 7, 15, 25, 30 and 50 day old were irradiated with 100, 200, 300 and 400r X-rays and then mated with normal females. 150 pregnant females were killed on the 19th day of gestation and about 1000 fetuses were obtained, among which only one was found to be dwarf. The rest of the fetuses were diagnosed as normal.

b. Malformation in mouse embryos derived from irradiated pregnant females: 1) Thirty females were irradiated by a single dose of X-rays, varying from 5r to 10r, one day after copulation and 200 newborn youngs which were obtained from such irradiated females were examined

in special regards to the formation of malformation. Results showed only 7 exencephalia had been induced.

2) Ten females were irradiated by a single dose of 150r of X-rays on the 12-13th day of gestation and 80 newborn youngs were obtained, among which about 25 percent showed an abnormal development of toes. These works are continued and in progress.

c. Sensitivity of immature mouse germ cells to X-rays administrated at various developmental stages: DM male mice aged 7, 15, 25, 30 and 50 days were irradiated through whole body exposure with doses of 100, 200, 300 and 400r of X-rays. Some genetic effects of irradiation on developmental stages of spermatogenesis were studied in females mated with irradiated males with special regard to pregnancy rate, mortality of embryos and mean litter-size. The results obtained are as follows: 1) Pregnancy rate of females which mated with irradiated males were nearly the same as that of females mated with untreated males. 2) Moles increased remarkably in number in the irradiated groups, especially in the group irradiated at the age of 25 days, being 13.8 percent in frequency. 3) Mean litter-size decreased slightly in the group irradiated at 25 days' age. On this basis, a possible suggestion was made that spermatocytes and spermatids are the most sensitive to X-rays.

d. Pregnancy and litter size of females mated with irradiated adult mice at various time in relation to the post-radiation effects: five DM mice being nearly 60 day-old were used for this study. Male adult mice were exposed to the 300r total-body irradiation of X-rays and were mated 10 times from just after the irradiation to 35 days later at about 4 days interval. Each male was placed together with a normal virgin female for mating at each time and in the next morning female was examined for the presence of vaginal plug, a sign of successful copulation. Mating rate in experiment group was rather low (75%) compared with the control (95%). As the results of observation on offsprings, litter size seemed to decrease gradually as the time between radiation and copulation, from 4.5 to 2.8 in average. The control showed 4.8. This result might suggest that injuries of germ cells by X-irradiation increase with the lapse of time. Further studies on this project are in progress.

4. EFFECTS OF X-RAYS ON THE SPERMATOGENESIS OF DROSOPHILA VIRILIS

The purpose of this study was to examine the radio-sensitivity of germ cells in various stages of spermatocytes in D-virilis. Works were continued from the former year, under two following items:

a. Emergence rate of pupae irradiated at various stages after pupation: With 2,000r of X-rays, 696 pupae were irradiated at various stages and the relationship was studied between the rate of emergence and the time they were irradiated. It was observed that none of pupae irradiated 20 hours or less after pupation yielded flies, while pupae irradiated 40 hours or more after pupation yielded flies at almost the same rate of emergence as non-irradiated pupae, with the exception of pupae irradiated 45 and 55 hours after pupation, which showed a slight decrease of the rate, as shown in the following table.

Table 4: Rate of Emergence
from Irradiated Pupae

Age of pupae irradiated after pupation	No. of pupae irradiated	No. of flies emerged			Rate of emergence
		Female	Male	Total	
5 (hrs.)	29	-	-	-	0 (%)
30	47	-	-	-	0
40	132	42	50	92	69.9
45	33	9	6	15	45.5
55	14	4	5	9	64.3
60	80	38	37	75	93.8
65	92	38	48	86	93.5
80	37	15	16	32	86.5
90	35	21	11	32	94.3
95	54	30	20	50	92.5
100	55	17	31	48	87.4
115	52	23	24	47	90.4
140	15	5	7	12	80.0
170	21	9	10	19	90.5
Total	696	252	265	517	74.3
Control	939	430	434	864	92.1

b. Cytological studies on the effects of X-irradiation on the germ cells of pupae in *Drosophila virilis*: Pupae were irradiated by 2,000r of X-rays at the periods of 40, 50, 60, 70, 80, 90, and 100 hours after the pupation. Spermatogonial metaphases of these irradiated pupae were observed by means of squosh preparations which were made at 5, 24, and 48 hours after irradiation. Chiefly, chromosome stickiness was observed at 5 hours after irradiation. More than fifty percent of the structural changes of chromosomes were observed at 24 and 48 hours after irradiation.

5. ^{GAMMA}EFFECTS OF γ -IRRADIATIONS ON MATING TYPE OF PARAME-
CIUM

The purpose of this study is to reveal a possible hidden biological effect of ~~X~~^{gamma}-rays which would not appear in one generation. Paramecia were irradiated with gamma-ray at a dose of 132 kr which was found in the previous study as the dose to induce no abnormal division of micronuclei, and were cultured until the time of conjugations, when any possible changes might occur in irradiated individuals would be examined by means of mating reaction with standard, non-irradiated individuals. As a preliminary experiment, in order to obtain the homozygotes prior to the irradiation, paramecia of stocks '64' (mating type I) and '32' (mating type II) were artificially passed through the autogamy. These stocks were isolated from the original clone of *P. polycarum* which was established in our laboratory and has the binary mating type. The first experiment was carried out in hope of determining which type of inheritance was taken place in mating behavior of paramecia. Animals of type I received 132 kr of gamma-irradiation from Cobalt 60 source, were mixed with the non-irradiated specimens which belonged to the opposite mating type. Then twenty pairs were isolated as true crosses. From these pairs, forty conjugants were singly isolated and transferred into culture medium. About twelve hours later, it was found that most of these animals divided only once. These animals were isolated into separate culture media again. After an additional fission, they were reisolated and transferred into the medium of test tube culture. Thus the clone of 136 caryonides which were descendants of 17 conjugating pairs were obtained. We could not approach the conclusion, however, these clones are very useful to analyse the genetical behavior. Further studies are in progress.

6. IMMUNOLOGICAL RESPONSE OF LETHALLY IRRADIATED MICE WHICH RECEIVED INJECTION OF HETEROLOGOUS SPLEEN CELLS

The project had been almost completed in the last year, however, it was required to assess the results more in detail in a study on some histological features of spleens in sublethally irradiated mice. Results are as follows: the following three groups of mice furnished the material; mice received a single irradiation (Group A), mice irradiated and injected rat spleen homogenates 4.5 hours after irradiation (Group B), mice received injection of rat spleen homogenates 6 days before irradiation (Group C). Gamma-rays were irradiated with the whole body exposure at a dose of 650r.

Histological remarks of spleens: By 2 days after irradiation, the most remarkable feature of the irradiated animals was both hypertrophy of splenic white pulp and red pulp atrophy. Small collections of lymphocytes along blood vessels in the white pulp were also very conspicuous in irradiated spleens. Large lymphocytes accumulated in the peripheral part of white pulp. These changes suggest some reactive changes occurring in spleens after irradiation.

With the lapse of time, in the spleens of the A group mice showed many pale staining reticular cells, among which massive collections of small lymphocytes distributed throughout the red pulp. It was striking that megakaryocytes developed in the red pulp of the irradiated mice of all groups. In the A group mice megakaryocytes appeared at first on 10th day after irradiation and increased in number with days until the death of animals. While in groups B and C, megakaryocytes were not observed until 20 days after irradiation. Therefore, the appearance of megakaryocytes in the red pulp of the irradiated animals seems to be a sign of myeloid metaplasia and of death of host animals. The spleens of the groups B and C showed almost similar reaction patterns. Within a few days after irradiation, marked accumulation of large lymphocytes occurred in the peripheral region of white pulp. Many basophilic cells were seen in the red pulp. Reticular cells were prominent, showing a heavy basophilic staining reaction in the cytoplasm. By 5 to 12 days after irradiation, the white pulp was replaced by large, pale staining lymphocytes. The appearance of some plasma cells, large reticuloendothelial cells and lymphoblasts was also remarkable in this state. By 30 days, the spleen resumed a normal appearance with normal germinal center.

7. EFFECTS OF X-RAYS ON THE PREGNANCY RATE IN MICE
RECEIVED POLYOVLATION TREATMENT

a. Induced polyovulation and super pregnancy in mature mice (Experiment I and II): these experiments were carried out as the control. The combined application of 5 i.u. pregnant mare's serum (PMS) and 5 i.u. human chorionic gonadotrophin (HCG) 44 hours apart (Experiment I), or that of 10 i.u. PMS and 5 i.u. HCG at the same interval (Experiment II), in mature female mice regardless of their oestrous cycle induced polyovulation in them. The number of ovulated eggs was significantly larger in Experiment I than in Experiment II. Generally, treated females carried fetuses in an usually large number, but some of the latter died or were resorbed during gestation. Some females gave birth to a large litter, but most of them produced a few or no living young.

b. Induced polyovulation in mature golden hamster: this work had been done as a supplemental experiment. Female golden hamsters at various stages of the oestrous cycle were injected with 20 i.u. PMS; the several groups further received an injection of HCG, 42, 44, 46, or 48 hours later respectively. In females treated at an interval of 46 hours, the number of ovulated eggs was significantly higher than that in the other three experimental groups. Eight amongst 10 treated females had copulated. In those eight females, the number of eggs extruded in the fallopian tube was 214 in total, varying from 18 to 37 in number, being 26.8 per individual. Microscopically those polyovulated eggs were normal in their general structure. Out of the fertilized eggs, 16.0 eggs per individual were found in process of early development, mostly being at the pronuclear stage and at the 2-cell stage of cleavage.

c. Effects of X-irradiation upon developing polyovulated mouse eggs: the effects of X-irradiation on developing polyovulated mouse eggs induced by hormones were observed, with special regard to the incidence of pregnancy and abnormalities of embryos and newborn young, in relation to the radioprotecting effect of endocrine treatment. X-ray irradiation was made with the following five doses: 10r, 25r, 50r, 100r, and 200r. The results obtained are as follows: 1) the irradiation of 25r was significantly effective in inducing preg-

nancy, resulting in a frequency of 67.9 percent, while 10r was not effective and 200r was injurious, 2) the average number of implants with normal appearance slightly increased after low level irradiation, but did not noticeably differ by dosages, 3) the mean litter size slightly decreased in experimental groups; and 4) the number of externally normal developing eggs was nearly the same in both control and experimental groups following 25r irradiation.

It was concluded that, in order to facilitate the implantation of hormone-induced polyovulated eggs, 25r X-irradiation is effective.

II. LIST OF PAPERS PUBLISHED AND IN PRESS

- Ohzu, E. 1962. Preliminary notes on sensitivity of immature mouse germ cells to X-rays administered at various developmental stages. Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 15:51-56.
- Sato, A. 1962a. Some observations on induced polyovulation and superpregnancy in mature mice. Embryologia 7:284-294.
- Sato, A. 1962b. Induction of superovulation and pregnancy in mature and immature rats by the application of gonadotrophins. Jap. J. Genet. 37:253-259.
- Sato, A. 1962c. Notes on induced polyovulation in mature golden hamsters. Jour. Fa. Sci. Hokkaido Univ. Ser. VI, Zool. 15:57-64.
- Sato, A. 1962d. On some effects of ionizing radiations upon developing polyovulated mouse eggs. Embryologia (in press)
- Yoshida, M. and S. Makino 1963. A chromosome study in a non-treated and an irradiated human in vitro cell line. Jap. J. Human Genet. (in press).

APPENDIX "A"

Table 1. Results of chromosome counts in a non-irradiated human cell line

Passage	Days after set up	Date	Chromosome number distribution						Tetraploid (%)
			44	45	46	47	90	92	
1	4	19-IV/61			21			1	4.7
2	9	23-IV/61		1	45				
3	14	28-IV/61			91				
4	20	4-V/61			31				
5	27	11-V/61			61				
6	32	16-V/61			49				
7	38	22-V/61			61				
8	45	29-V/61			60				
9	49	2-VI/61			21				
10	57	10-VI/61			31				
11	63	16-VI/61			19				
12	71	23-VI/61		1	35				
13	77	29-VI/61			45				
14	86	8-VII/61			62				
15	93	15-VII/61			38				
16	100	21-VII/61			50				
17	106	27-VII/61		1	68				
18	111	1-VIII/61			61				
19	118	8-VIII/61			65				
20	125	15-VIII/61		1	69	1			
21	131	21-VIII/61			71				
22	138	28-VIII/61		1	77				
25	156	15-IX/61		1	65				
29	177	6-X/61			59				
35	201	30-X/61			55			1	1.8
40	273	10-1/62		1	51			1	1.9
41	284	21-1/62			38				
45	323	1-III/62		1(3)	41	2		7(2)*	16.0
46	331	9-III/62		(8)	93	1	(1)	7(6)*	12.0
47	340	18-III/62	2	2(7)	163	1	(4)	17(5)*	20.9

Numbers in parentheses represent cells with dicentric chromosome.
(*) indicates number of cells showing endoreduplication.

APPENDIX "B"

Table 2. Chromosome conditions in an irradiated human cell line (200r, X-rays)

Passage	Days after irradiation	Chromosome number distribution							Cells with chromosome abnormalities (per cent)	Polyploid
		44	< 44	45	46	47	48	> 48		
1	7	1	4	3	56	1			9(12.0%)	6(8.0%)
2	14			12	66	5			4(4.6%)	8(8.7%)
3	24			9	33					
4	31			2	21	3				
5	49			1	71	1				
6	59			1	98	1				
7	62		3	2	99	3		2		3(2.6%)
8	69	1	1		69		2			
9	76	1			21	1				1(4.1%)
10	84			1	62	1				1(1.5%)
11	92			1	31					1(5.0%)
12	101				45	1				

APPENDIX "C"

Table 3. Number of cells with chromosome injuries
in serially irradiated cell line.

Irradiated groups	No. of cells observed	No. of cells unaffected	No. of cells injured
Group-R-I	50	13(26%)	37(74%)
Group-R-II	74	33(44.5%)	41(55.5%)
Group-R-III	50	29(58%)	21(42%)
Group-R-IV	60	35(58.3%)	25(41.7%)
Group-R-V	61	35(57.3%)	26(42.7%)
Group-R-VI	65	49(76.1%)	16(23.9%)